

***Ganoderma multipileum* and *Tomophagus cattienensis*— new records from Pakistan**

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ABSTRACT—New records of *Ganoderma multipileum* and *Tomophagus cattienensis* collected from Changa Manga Forest and Lahore, Pakistan, are presented based on morphological and nuclear rDNA ITS sequence data from fresh basidiomata. Specimens previously treated as *G. lucidum* from Pakistan were reviewed and found to represent different species, among them *G. multipileum*. Specimens of *T. cattienensis* determined for the first time from Pakistan presented morphological features similar to *T. colossus* but corresponded molecularly to *T. cattienensis*; the morphological description for *T. cattienensis* is expanded accordingly.

KEY WORDS—*Ganodermataceae*, morphology, Punjab, taxonomy

Introduction

Ganodermataceae, a monophyletic polypore family with ganodermatoid basidiospores (Costa-Rezende & al. 2020) previously included in *Polyporaceae* (Justo & al. 2017, He & al. 2019), is characterized by its shelf-like basidiomes, poroid hymenophores, and complex-walled basidiospores. *Amauroderma* Murrill, *Amaurodermellus* Costa-Rezende & al., *Cristataspora* Robledo & Costa-Rezende, *Foraminispora* Robledo & al., *Furtadoa* Costa-Rezende & al., *Ganoderma* P. Karst., *Haddowia* Steyaert, *Humphreya* Steyaert, and *Tomophagus* Murrill are some genera currently included in *Ganodermataceae* (Murrill 1905; Moncalvo 1996; Moncalvo &

Ryvarden 1997; Ryvarden 2004; Kirk & al. 2008; Costa-Rezende & al. 2017, 2020).

Several species of fungi with ganodermatoid basidiospores (specifically in *Ganoderma*) have been used as medicine in Asia for more than 2000 years where they have been cultivated on an industrial scale (Wasser & Weis 1997). Despite of their significance, this group of fungi remains poorly studied in many regions of the world such as in Pakistan, where *Ganoderma ahmadii* Steyaert, *G. applanatum* (Pers.) Pat., *G. australe* (Fr.) Pat., *G. boninense* Pat., *G. chalconum* (Cooke) Steyaert, *G. curtisii* (Berk.) Murrill, *G. flexipes* Pat., *G. lipsiense* (Batsch) G.F. Atk., *G. lucidum* (Curtis) P. Karst., *G. perzonatum* Murrill, *G. philippii* (Sacc.) Bres., *G. praelongum* Murrill, *G. multicornum* Ryvarden, *G. multiplicatum* (Mont.) Pat., *G. resinaceum* Boud., *G. tornatum* (Pers.) Bres., *G. tsugae* Murrill, and *Tomophagus colossus* (Fr.) Murrill [$\equiv$ *G. colossus* (Fr.) C.F. Baker] have been recorded (Ahmad 1956, 1972, Steyaert 1972, Irshad & al. 2012, Fakhar-ud-Din & Mukhtar 2019). These species were determined primarily based on morphological concepts; however, there is much uncertainty regarding which morphological criteria should be used to assign species to *Ganodermataceae* and specifically to *Ganoderma* (Moncalvo & Ryvarden 1997, Welti & Courtecuisse 2010).

Clarification on the placement of species in *Ganoderma* or related genera is essential, not only for taxonomic reasons, but also to investigate the economical and pharmacological importance of the species that actually grow in the tropics. The aim of this study was to use morphological and molecular analyses to show that some specimens previously identified as *G. lucidum* in Pakistan actually represent *G. multipileum*, reported here as a first record for the country. We also report *Tomophagus cattienensis* from Pakistan for the first time.

Materials & methods

Collections & ecology

Fungal specimens were collected in Punjab, Pakistan, during 2018 by the first author and deposited in the Herbarium of the Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH). The material was collected in [1] Changa Manga Forest in the Kasur District, dominated by *Dalbergia sissoo* DC. and *Vachellia nilotica* (L.) P.J.H. Hurter & Mabb. (*Fabaceae*) and with an average annual rainfall of 1232 mm and average 24 °C temperature (Ahmad & al. 2014) and [2] the New Campus, University of the Punjab in Lahore, covered by *D. sissoo* with many rotting trunks and with an average annual rainfall of 607 mm and average 24 °C temperature (Shirazi & al. 2019). Additionally, a specimen from XAL was re-examined.

TABLE 1. Sequences of *Ganoderma*, *Tomophagus*, and *Perenniporiella* outgroup included in the analyses. [T] ex-type sequences.

SPECIES	VOUCHER/STRAIN	LOCALITY	GENBANK ITS1/ITS2	REFERENCE
<i>G. ahmadii</i>	FWP 14329 [T]	Pakistan	Z37047/ Z37098	Moncalvo & al. 1995
<i>G. curtisii</i>	CBS 100132	USA	JQ781848	Cao & al. 2012
	UMNGA1	USA	MG654117	Loyd & al. 2018
<i>G. leucocontextum</i>	AY2B	Pakistan	MN134012	Unpublished
	GDGM 40400 [T]	China	KF011548	Li & al. 2015
<i>G. lingzhi</i>	Cui 9166(67)	China	MH109560	Unpublished
	Dai 12574	China	KJ143908	Zhou & al. 2015
<i>G. lucidum</i>	K 175217	UK	KJ14391	Zhou & al. 2015
	MUCL 35119	France	MK554779	Cabarroí-Hernández & al. 2019
	CWN01740	Taiwan	EU021461	Wang & al. 2009
	BCRC36123	India	EU021459	Wang & al. 2009
<i>G. martinicense</i>	LIP SW-Mart08-44/ MUCL:GSP44	Martinique	KF963257	Welti & al. 2015
	LIP SW-Mart08-55/ MUCL:GSP55 [T]	Martinique	KF963256	Welti & al. 2015
<i>G. mizoramense</i>	UMN-MZ5	India	KY643751	Crous & al. 2017
	UMN-MZ4	India	KY643750	Crous & al. 2017
<i>G. multipileum</i>	CM10	Pakistan	MW349830	This study
	CM110	Pakistan	MW349829	This study
	CWN 04670	China	KJ143913	Zhou & al. 2015
	Dai 9447	China	KJ143914	Zhou & al. 2015
	HMAS242384	China	JF915409	Wang & al. 2012
<i>G. multiplicatum</i>	MN14091107	Myanmar	MK345439	Hapuarachchi & al. 2019
	Dai 13710	China	KU572489	Unpublished
	Dai 12320	China	K00U572490	Unpublished
	JFL 10004081328	China	MH106879	Hapuarachchi & al. 2018
	SPC9	Brazil	KU569553	Bolaños & al. 2016
	SPC5	Brazil	KU569549	Bolaños & al. 2016
<i>G. parvulum</i>	MUCL 47096	Cuba	MK554783	Cabarroí-Hernández & al. 2019
	MUCL 53123	French Guiana	MK531814	Cabarroí-Hernández & al. 2019
	URM80765	Brazil	JX310822	De Lima Jr & al. 2014
	URM2948	Brazil	JX310821	De Lima Jr & al. 2014

SPECIES	VOUCHER/STRAIN	LOCALITY	GENBANK ITS1/ITS2	REFERENCE
<i>G. resinaceum</i>	CBS 194.76	Netherlan0ds	KJ143916	Zhou & al. 2015
	MUCL 52253	France	MK554786	Cabarroi-Hernández & al. 2019
<i>G. sichuanense</i>	HMAS251146	China	JF915401	Wang & al. 2012
<i>G. valesiacum</i>	CBS 282.33	UK	Z37081/ Z37056	Moncalvo & al. 1995
<i>Ganoderma</i> sp.	AU-2019a/HP12	Pakistan	MN006955	Unpublished
<i>P. chaquenia</i>	MUCL 47648	Argentina	FJ411084	Robledo & al. 2009
<i>P. pendula</i>	MUCL 47129	Cuba	FJ411082	Robledo & al. 2009
<i>T. cattienensis</i>	CATPU120	Pakistan	MW737424	This study
	CATPU121	Pakistan	MW737425	This study
	CT119	Vietnam	JN184398	Le & al. 2012
	CT99 [T]	Vietnam	JN184397	Le & al. 2012
<i>T. colossus</i>	255FL	USA	MG654427	Loyd & al. 2018
	CGMCC5.763	Philippines	JQ081068	Wang & al. 2012
	UMNFL151	USA	MG654431	Loyd & al. 2018
	URM80450	Brazil	JX310825	De Lima Jr & al. 2014
	URM83330	Brazil	JQ618247	De Lima Jr & al. 2014
	TC-02	Vietnam	KJ143923	Zhou & al. 2015
<i>Tomophagus</i> sp.	BAB-4989	India	KR155077	Unpublished
	AUMC 14536	Egypt	MW186858	Unpublished

Morphology

Size, shape, and color of basidiomata were noted from fresh material. Color descriptions follow Munsell (1975). For microscopical analyses, basidiome cross sections were soaked in 3% KOH, stained with 1% Congo red, and examined under 100× magnification using a Meiji MX4300H compound light microscope. At least 30 basidiospore measurements (face and side view, excluding the apical umbo) were recorded and rounded to the nearest 0.5 μm; dimensions are presented as length × width (Nagy & al. 2010). Microscopical terms follow, in part, Torres-Torres & Guzmán-Dávalos (2012). Color descriptions follow Munsell (1975).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from dried specimens following a modified CTAB procedure (Doyle & Doyle 1987). The ITS1+5.8S+ITS2 rDNA region (ITS) was amplified using primers ITS1 & ITS2 (White & al. 1990). Reaction mixtures (20 μl) containing 0.5 μl template DNA, 8.5 ml distilled water, 0.5 μl of each primer, and 10 ml DreamTaqGreen PCR Master Mix (2 X) ran 35 cycles of 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. The PCR amplicons were purified and sequenced by Tsingke Co. Ltd. (Tianjin, China)

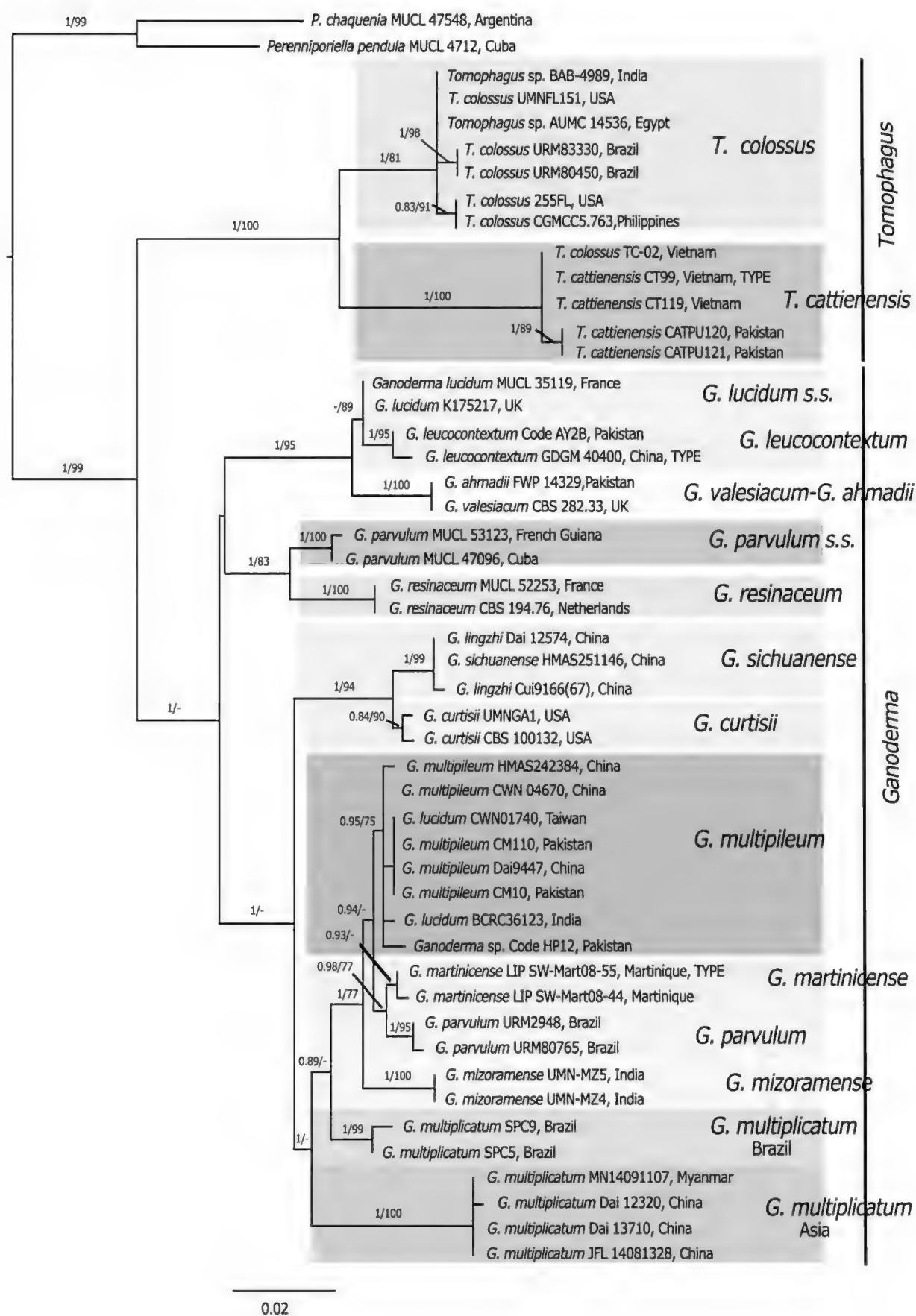


FIG. 1. Phylogenetic tree of *Ganoderma* and *Tomophagus* based on ITS rDNA sequences generated by maximum likelihood. *Perenniporiella pendula* and *P. chaquenia* were chosen as outgroup. Posterior probabilities (>0.85) and bootstrap values (>75%) are shown at the branches.

Phylogenetic analysis

The data set comprised four DNA sequences from Pakistani material and 45 ITS sequences downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/) (TABLE 1). The ITS data set was subdivided into three parts: ITS1, 5.8S, and ITS2. *Perenniporiella chaquenia* Robledo & Decock and *P. pendula* Decock & Ryvarden were selected as outgroup (Costa-Rezende & al. 2017).

All sequences were automatically aligned with MUSCLE (Edgar 2004) and manually adjusted using PhyDe® (Müller & al. 2010). JModelTest (Posada & Crandall 1998) was used to determine the best evolutionary model using the corrected Akaike information criterion (AICc). Maximum Likelihood (ML) analyses were conducted using RAxML 7.0.4 (Stamatakis 2006) and Bayesian Inference (BI) analyses were conducted using MrBayes v.3.2.2 (Ronquist & Huelsenbeck 2003). In the ML analysis, the default priors were used, performing 1000 replicates under the GTRGAMMA model. BI analyses were run on CIPRES Science Gateway (Miller & al. 2010). Two independent runs, with 2,000,000 generations each, were carried out with a sampling frequency every 1000 generations and a burn-in of 25%. A 50% majority rule consensus tree with posterior probabilities (PP) was obtained. Convergence of the Markov chains to a stationary distribution was assessed by visual examination of the log likelihood values in the program Tracer v1.7.1 (Rambaut & al. 2018). Nodes were considered supported when bootstrap values (BS) were $\geq 75\%$ and the PP was ≥ 0.85 . The final alignments were deposited in TreeBASE (www.treebase.org).

Phylogenetic results

The evolutionary models that best fit the individual dataset according to the AICc were ITS1 = GTR+G, 5.8S = K80, ITS2 = GTR+G. In BI analyses, the average standard deviation of split frequencies was 0.007460. Phylogenetic analysis of the ITS region indicated that our specimens represent *Ganoderma multipileum* and *Tomophagus cattienensis* (FIG. 1). All sequences of *G. multipileum*, including the Pakistani collections CM10 and CM110, are supported in one clade (PP = 0.95; BS = 75%). This clade, which included sequences of specimens from China, India, and Taiwan, also clustered with sequences from “*G. lucidum*” specimens and formed a sister group with *G. martinicense* Welte & Courtec. and *G. parvulum* Murrill (0.94 PP; 68% BS). The other two Pakistani sequences (from CATPU120 and CATPU121) clustered in the strongly supported *Tomophagus* group (PP = 1; BS = 100). The Pakistani sequences and the holotype of *T. cattienensis* (CT99) formed a monophyletic group with another *T. cattienensis* sequence from Vietnam and sequence TC-02 (as *T. colossus*, surely a misdetermination), also from Vietnam.

Taxonomy

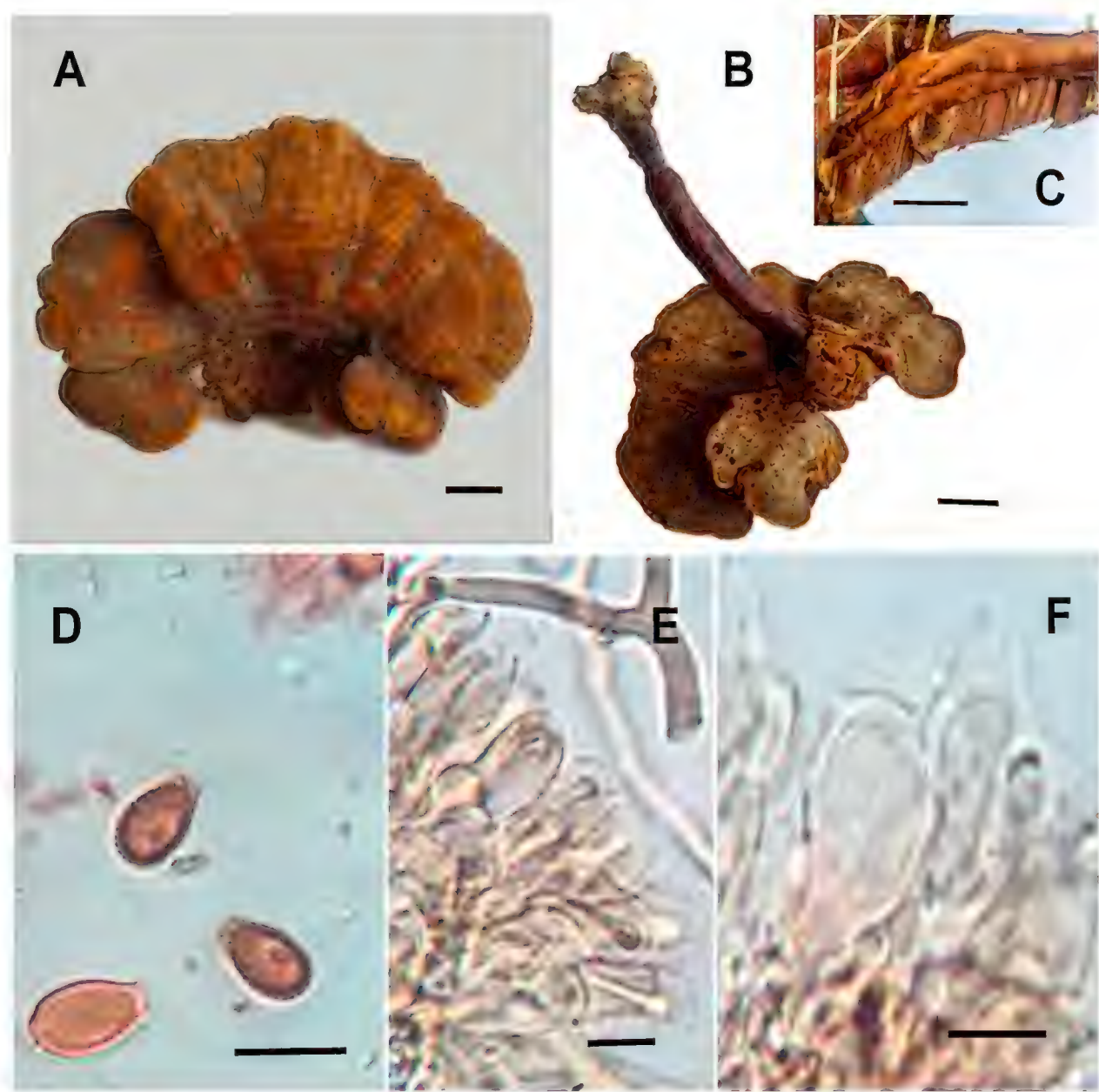


FIG. 2. *Ganoderma multipileum* (LAH36825). A. Pileus; B. Pore surface and stipe; C. Section of context and tubes; D. Basidiospores; E. Cells of crustohymeniderm; F. Basidium. Scale bars: A–C = 2 cm; D–F = 10 μ m.

Ganoderma multipileum Ding Hou [as ‘*multipilea*’],
Quart. J. Taiwan Mus. 3: 101 (1950)

FIG. 2

BASIDIOMATA annual; stipitate, solitary, single or with a group of 3–4 pilei growing from the same stipe, light in weight, corky. PILEUS projecting ≤ 12 cm, 12.2 cm wide, and ≤ 2.9 cm thick at the base; reniform, dimidiate to flabelliform, laccate, sulcate, with concentric growing zones; crust thin, yellowish brown (2.5YR4/8) to brown (10R4/6); MARGIN 0.2–0.3 cm thick, entire, obtuse, orange-brown (10YR6/8) to brown (5YR 5/8). STIPE 9–9.6 $\times$

3–4.1 cm, sub-cylindrical, eccentric to lateral, strongly laccate, woody, maroon-brown (2.5YR3/6). PORES 6–7 per mm, 110–140 µm diam., sub-round to round, straw cream (7.5YR8/4) to brown when bruised (2.5YR4/6). TUBES ≤1.3 cm long, brown (10YR4/6) to pale brown (2.5YR 8/4). CONTEXT ≤1.7 cm thick, with concentric growth zones, brown (7.5YR6/8) to yellowish-brown (5YR 5/8), with melanoid incrustations.

HYPHAL SYSTEM DIMITIC: 1) generative hyphae 3.5–5 µm diam., thin-walled, hyaline, clamped; 2) arboriform skeleto-binding hyphae 2.5–7 µm diam., thick-walled, red-brown to yellow brown. PILEIPELLIS a crustohymeniderm; cells 15–60 × 4.5–12.5 µm, clavate, thick-walled. BASIDIA 20.5–29 × 5.5–8.5 µm, claviform, hyaline. BASIDIOSPORES 8.0–13.2 × 5.5–7.4 µm, ellipsoid to ovoid, apex truncate, brown, double walled with endosporic ornamentation as solid, thin, free pillars or column-like projections.

SPECIMENS EXAMINED—PAKISTAN, PUNJAB, Kasur district, Changa Manga Forest, 31.05°N 73.4072°E, 200 m a.s.l., gregarious on decayed hardwood, *Dalbergia sissoo* and *Vachellia nilotica*, 10 July 2018, Aisha Umar CM10 (LAH36826, GenBank MW349830). Lahore district, Lahore, New Campus, University of the Punjab, 31.4981°N 73.3044°E, 217 m a.s.l., gregarious on hardwood, on living tree trunk of *V. nilotica*, 10 April 2018, Aisha Umar CM110 (LAH36825, GenBank MW349829).

DISTRIBUTION: China, India, Nepal, Pakistan, Philippines, Taiwan, Thailand.

Tomophagus cattienensis X.T. Le & Moncalvo,

Mycol. Prog. 11: 777 (2012)

FIG. 3A–E

BASIDIOMATA annual, sessile, bulky, light in weight, spongy. PILEUS projecting ≤9.2 cm, 10.2 cm wide, and ≤7.8 cm thick at the base, dimidiate to flabelliform, laccate, surface friable, rugose, golden (10YR7/8), yellow-brown (10YR8/8), orange-brown (10YR6/8), colors patchy and not on a gradient; MARGIN 3.4–4 cm, very thick, entire to lobulated, obtuse, rugose, whitish, white creamy (10YR8/3) to pale yellow brown (10YR7/6). PORES 2–3 (–4) per mm, angular to round, creamy (7.7YR8/4) to brown (10YR3/4) when bruised. TUBES 0.9–1.2 cm long., golden yellow (10YR7/8). CONTEXT 3.8–7.6 cm thick, thick at the base, homogeneous, light, soft, creamy white (2.5YR8/4) when fresh to pale brown (10YR4/6) when dry, powdery.

HYPHAL SYSTEM DIMITIC: 1) generative hyphae 2.5–3 µm diam., thin-walled, clamped, hyaline; 2) skeletal hyphae 2–3 µm diam., thick-walled, hyaline. PILEIPELLIS a crustohymeniderm; cells 40–80 × 7–15 µm, narrowly clavate, thick-walled, apically ornamented. CHLAMYDOSPORES 22–24.5 µm diam., globose, double walled, endosporic ornamentation with thick cylindrical projections, yellowish brown. BASIDIA not seen. BASIDIOSPORES

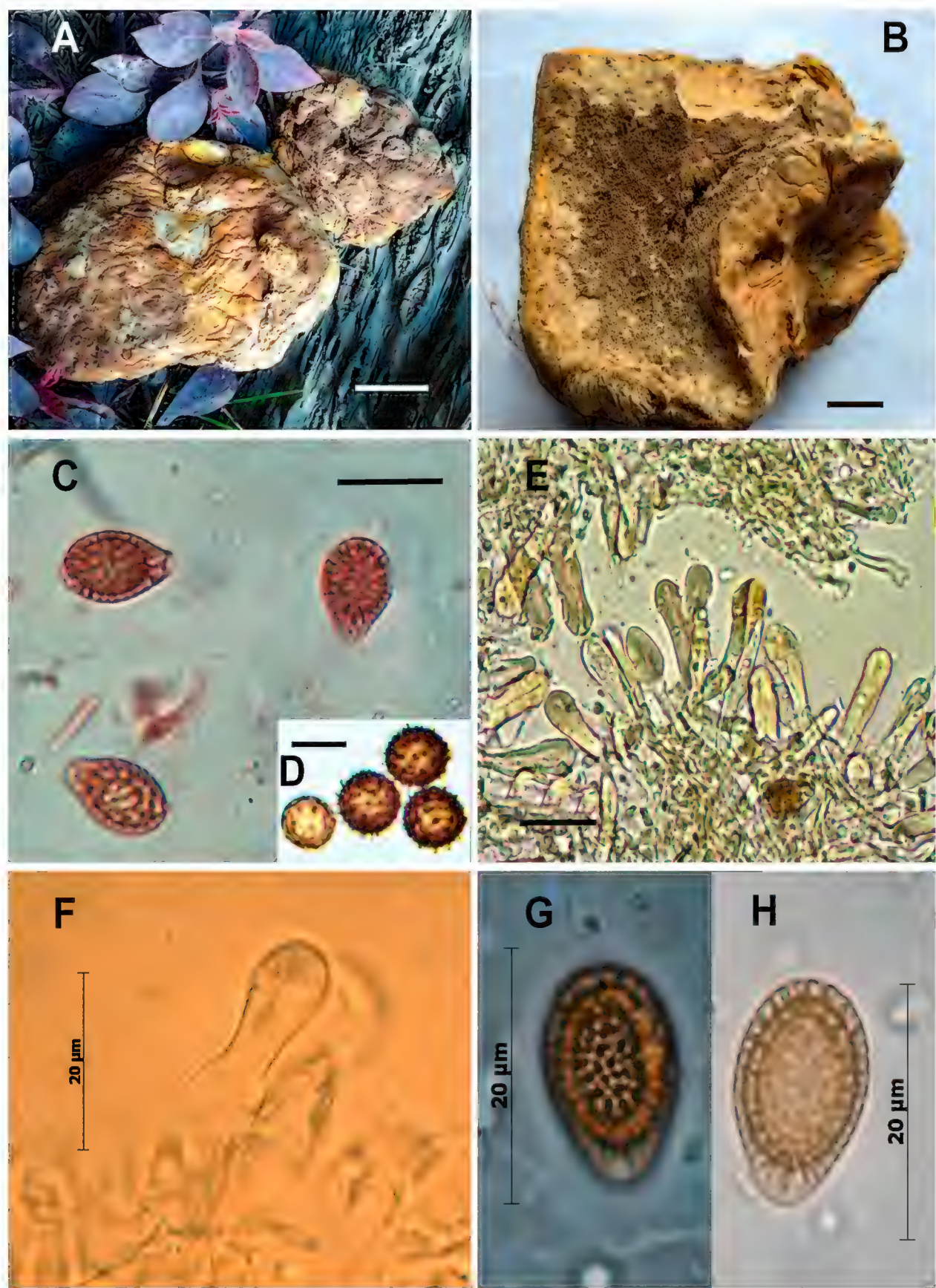


FIG. 3. *Tomophagus cattienensis* (LAH36830). A. Pileus; B. Pore surface and context; C. Basidiospores; D. Chlamydospores; E. Crustohymeniderm cells. *Tomophagus colossus* (XAL-Guzmán 35708). F. Crustohymeniderm cell; G, H. Basidiospores. Scale bars: A = 2 cm; B = 1 cm; C, D = 20 µm.

17.4–21.3 × 11.3–14.6 µm, ellipsoid to ovoid, apex acute or subacute, or when collapsed then shortly truncated or even concave, reddish brown, double-walled with endosporic ornamentation as thick, partially reticulate pillars, apex hyaline.

SPECIMENS EXAMINED—PAKISTAN, PUNJAB, Lahore district, Lahore, New Campus, University of the Punjab, 31.4981°N 73.3044°E, 217 m a.s.l., gregarious on hardwood, on living tree trunk of *Dalbergia sissoo*, 10 April 2018, Aisha Umar CATPU120 (LAH36830, GenBank MW737424). Kasur district, Changa Manga Forest, 31.05°N 73.4072°E, 200 m a.s.l., gregarious on decayed hardwood, *Vachellia nilotica*, 10 July 2018, Aisha Umar CATPU121 (LAH36839, GenBank MW737425).

DISTRIBUTION: Pakistan, Vietnam.

ADDITIONAL SPECIMEN EXAMINED (FIG. 3F–H)—*Tomophagus colossus*: MEXICO, VERACRUZ, Guzmán 35708 (XAL) [as *Ganoderma* “colossum”].

Discussion

Our morphological and molecular analyses confirm the presence of two polypores of *Ganodermataceae* from Pakistan: *Ganoderma multipileum* and *Tomophagus cattienensis*.

For many years, the name *Ganoderma lucidum* has been misapplied to *Ganoderma* specimens from tropical Asia that represent other species (Wang & al. 2009, Hennicke & al. 2016, Raja & al. 2017). Collections labeled as ‘*G. lucidum*’ from world regions outside Europe (where *G. lucidum* was described) have appeared in several different lineages in numerous phylogenetic analyses (Moncalvo & al. 1995, Gottlieb & al. 2000, Smith & Sivasithamparam 2000, Hong & Jung 2004). *Ganoderma multipileum* is one species confused with *G. lucidum* that is now recognized as representing a different species (Wang & al. 2009) first described over a half century ago by Hou (1950).

Lloyd & al. (2018) showed that the “multipileum clade” is sister to the “curtisii clade,” with the “multipileum clade” split into two subclades, one clustering specimens of *G. multipileum* from China and the other clustering specimens of *G. martinicense* from Martinique (type specimen) and from North America. *Ganoderma multipileum*, originally described from Taiwan (Wang & al. 2009), has been recorded from India, China, Nepal, and Philippines (Wang & al. 2012, Fryssouli & al. 2020). Welti & Courtecuisse (2010) suggested that *G. martinicense* might represent a “vicariant from the Caribbean area” of *G. multipileum*. Our phylogeny, although based on a single DNA marker, agrees with that of Lloyd & al. (2018) based on four markers and supports a relationship between *G. multipileum* and *G. martinicense*.

However, our phylogeny also includes two sequences of *G. parvulum* (De Lima Jr & al. 2014) as sister taxon of *G. martinicense*. Cabarroi-Hernández & al. (2019) suggested that the sequences of *G. parvulum* sensu De Lima Jr. & al. (2014) might represent *G. bibadiostriatum* Steyaert, a species described from Nicaragua (Steyaert 1962) and phylogenetically unrelated to *G. parvulum* s.str. (Cabarroi-Hernández & al. 2019).

Our “multipileum clade” comprises sequences of specimens from China, India, and Taiwan and CM10 and CM110 (previously labeled *G. lucidum*) from Pakistan. The clade also includes the sequences BCRC36123 from India and CWN01740 from Taiwan, both referenced at GenBank as *G. lucidum* but identified as *G. multipileum* by Wang & al. (2009), as well as the sequence Code HP12 referenced at GenBank as *Ganoderma* sp. from Pakistan (very probably representing *G. multipileum*). The ITS tree corroborates our morphology-based identification and supports extending the distribution of *G. multipileum* to Pakistan. Furthermore, we suggest that *G. lucidum* s.str. is not present in that country.

Wang & al. (2009) concluded that, based on morphological characters and their ITS phylogeny, “*G. lucidum*” from tropical Asia is divided into two clades, both of separated from the European *G. lucidum* s.str. One clade contained tropical collections (labeled *G. multipileum*) while a second “unknown” clade clustered specimens from China and Japan (Wang & al. 2009). Wang & al. (2012) later recognized the “unknown” clade as *G. sichuanense* J.D. Zhao & X.Q. Zhang, while Cao & al. (2012) noted that *G. multipileum* also occurred in India and the Philippines.

Ganoderma multipileum is recognized morphologically based on three features: basidiomes with pilei or with some stipes and pilei growing together, mostly regular clavate crustohymeniderm cells, and truncated ellipsoid basidiospores with free fine pillars (Wang & al. 2009, Zhou & al. 2015). According to Cao & al. (2012) *G. multipileum* inhabits fabaceous hosts and is distinguished from *G. sichuanense* (as *G. lingzhi* Sheng H. Wu & al.) by its “distinct concentric growth zones in context at maturity, and finely echinulate basidiospores.” These primary diagnostic features are also seen in the Pakistani specimens of *G. multipileum* (FIG. 2). Furthermore, our specimens were collected growing gregariously on decayed hardwood of *Dalbergia* and *Vachellia* (Fabaceae).

The other Pakistani specimens studied here nested in the *Tomophagus* clade. Murrill (1905), who proposed the genus, characterized *Tomophagus* by its very lightweight basidiome with a pale soft spongy context and a

“labyrinthine” basidiospore surface (Le & al. 2012), as noted in both species covered here (FIG. 3C,G,H). *Tomophagus* was typified by *Polyporus colossus* Fr. (Murrill 1905). Furtado (1962, 1965), who described the neotype specimen as having “*Ganoderma*-type basidiospores,” accepted the species as *G. colossus*.

Moncalvo’s (2000) phylogeny revealed an unclassified basal group composed of *G. colossus*, *G. tsunodae* (Lloyd) Sacc. & Trotter, and other taxa. Subsequent molecular phylogenetic studies confirmed the genus as a well-established group independent of *Ganoderma* (Hong & Jung 2004, Le & al. 2012, Xing & al. 2018, He & al. 2019, Costa-Rezende et al 2017, 2020).

Tomophagus cattienensis and *T. colossus* have been distinguished primarily by color of the pileal surface and ITS sequence analyses (Le & al. 2012). *Tomophagus cattienensis* was distinguished from *T. colossus* by its “red-brown or red-coffee” (instead of yellow) pileal surface and by its harder crust and context that “turns pale brown upon drying (instead of remaining creamy white)” (Le & al. 2012). However, Pakistani specimens have yellow, yellow-brown, orange-brown pilei and lighter crusts (somewhat similar to *T. colossus* or intermediate between the two species) whereas the color change of the context agrees with that described for *T. cattienensis* (FIG. 2A). Another character distinguishing *T. cattienensis* and *T. colossus* are the thick-walled crustohymeniderm cells, present in the Pakistani specimens of *T. cattienensis* but not previously described for this species. *Tomophagus colossus* characteristically has thin-walled cuticular cells (Ryvarden 2000) observed in the specimen of *T. colossus* from Mexico (Guzmán 35708, XAL) (Torres-Torres & al. 2015).

Our observations of the Pakistani specimens therefore expand the morphological description of *T. cattienensis*. In our phylogeny, the ITS sequences of the Pakistani specimens showed only one nucleotide difference from ITS sequences of the *T. cattienensis* type specimen in contrast to the six-nucleotide difference with *T. colossus*.

Our phylogeny included sequences labelled *G. multiplicatum* in GenBank, which separated into two different clades. One clade (PP 1/BS 100) clustered specimens from China and Myanmar, and the other (PP 1/BS 99) clustered specimens from Brazil. Morphologically, *G. multiplicatum* is distinguished from *G. multipileum* primarily by its cuticular cells with up to 14 lateral or apical protuberances (Torres-Torres & Guzmán-Dávalos 2012, Bolaños & al. 2016). Originally described from French Guyana, *G. multiplicatum* was subsequently found in other parts of South America, Africa, and Asia (Steyaert 1980, Zhao 1989), including India (Bhosle & al. 2010), Pakistan

(Fakhar-ud-Din & Mukhtar 2019), and Taiwan (Wang & Wu 2008). The Asian specimens identified as *G. multiplicatum* should be re-evaluated and more DNA regions must be included, to define their taxonomical status.

In this study, we combined macro and microscopic morphological characters, ecological aspects, as well as molecular data to determine two species of *Ganodermataceae* from Pakistan. This is the first report of the occurrence of *G. multipileum* and *T. cattienensis* in this country. Our results suggest that some species with ganodermatoid basidiospores previously registered from Pakistan (e.g., *G. ahmadii*, *G. applanatum*, *G. australe*, *G. boninense*, *G. chalceum*, *G. curtisii*, *G. flexipes*, *G. lipsiense*, *G. lucidum*, *G. praelongum*, *G. multicornum*, *G. multiplicatum*, *G. resinaceum*, *G. tornatum*, *G. tsugae*, and *T. colossus*) need thorough re-evaluation of both morphology and molecular data before their presence in the country or taxonomic status can be confirmed.

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